Measuring P450-Glo® Assays on the GloMax® Discover System

Promega Corporation

Materials Required

- P450-Glo™ CYP3A4 Screening System (Luciferin-PPXE) (Cat.# V9910)
- P450-Glo™ CYP2C9 Screening System (Cat.# V9790)
  Note: both Screening Systems include recombinant CYP enzyme, enzyme substrate, control membranes, buffers, NADPH regenerating system components, Luciferin-free water, Luciferin Detection Reagent and Reconstitution Buffer.
- GloMax® Discover System (Cat.# GM3000)
- White, 96-well assay plate (Corning 3912)

Caution: We recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents.

Protocols: The *GloMax® Discover System Technical Manual* #TM397 is available at: **www.promega.com/protocols/**

The GloMax® Discover System in combination with the P450-Glo™ Assays provides a convenient, rapid and sensitive luminescent method to measure cytochrome P450 (CYP) activity. The P450-Glo™ Assays measure the activities of CYP enzymes from recombinant and native sources and can be used to test the effect of drugs and new chemical entities on CYP activity. All the P450-Glo™ Assays can be used for cell-free CYP inhibition studies, and many also can be used for cell-based CYP induction assays. The P450-Glo™ Substrates are CYP enzyme substrates that are derivatives of beetle luciferin [(4S)-4,5-dihydro-2-(6´-hydroxy-2´-benzothiazolyl)-4-thiazolecarboxylic acid]. They are converted to luciferin products by CYP enzymes. D-luciferin is formed and detected in a second reaction with the Luciferin Detection Reagent. The amount of light produced in the second reaction is proportional to CYP activity (Figure 1).

The P450-Glo™ Assays are easy to perform on the GloMax® Discover System, and the protocol comes pre-loaded on the instrument. The extended dynamic range and limited well-to-well cross-talk of the GloMax® Discover System allows you to easily measure various sample signal intensities on the same plate using the P450-Glo™ Assays. This Application Note describes the protocol for measuring luminescence using the GloMax® Discover System with the P450-Glo™ CYP3A4 (Luciferin-PPXE) and CYP2C9 Assays.

Protocol

1. Prepare 4X Enzyme/Substrate mixes containing the appropriate enzymes, and substrates for each CYP assay, buffer and water.
2. Prepare 4X enzyme diluent for each assay containing the CYP substrate, buffer and water.
3. Perform a serial twofold titration of each enzyme in its respective diluent.
4. Prepare 2X NADPH Regeneration System for each enzyme.
5. Transfer 12.5µl of the CYP2C9 enzyme titration to rows A-D of a 96-well assay plate.
6. Transfer 12.5µl of the CYP3A4 enzyme titration to rows E-H of the same 96-well assay plate.
7. Transfer 12.5µl of luciferin-free water to every well of the assay plate.
8. Transfer 25µl of NADPH Regenerating System to the enzyme wells.
9. Shake the plate on an orbital shaker for 30 seconds.

10. Cover the assay plate and incubate at room temperature for 30 minutes.
11. Prepare Luciferin Detection Reagent for each enzyme by thawing and combining Reconstitution Buffer with lyophilized luciferase enzyme. Invert to mix, and equilibrate to room temperature before use.
12. Transfer 50µl of Luciferin Detection Reagent to the assay wells for each enzyme.
13. Shake the plate on an orbital shaker for 30 seconds.
14. Incubate the assay plate at room temperature for 20 minutes.
15. Measure luminescence (CYP450 activity) using the GloMax® Discover P450-Glo™ protocol.

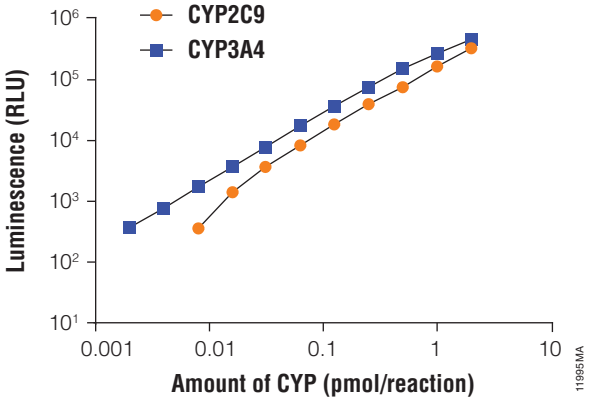

Figure 1. CYP activity is directly proportional to luminescent output. Serial twofold dilutions of CYP2C9 and CYP3A4 enzymes were performed. 12.5µl of each dilution was added to rows A-D and E-H, respectively, of a 96-well assay plate. Luciferin-free water (12.5µl) and 25µl of 2X NADPH Regenerating System were transferred to every well and the assay plate was incubated for 30 minutes at room temperature. 50µl of reconstituted Luciferin Detection Reagent was then added to the assay wells. After a 20-minute, room-temperature incubation, luminescence was measured using the GloMax® Discover System and the P450-Glo™ protocol.

GloMax® Discover System

The GloMax® Discover System offers superior sensitivity, dynamic range and limited well-to-well cross-talk. The instrument has been developed and optimized with Promega's industry leading Cell and Gene Reporter assays and may be integrated into low- and medium-throughput automation workflows. The GloMax® Discover System also provides flexible use of filters for fluorescence intensity, filtered luminescence, BRET, FRET, and UV-visible absorbance measurements for adaptation into a wide variety of laboratory applications. The instrument is operated by an integrated Tablet PC, which provides quick and easy navigation through the control options. Exporting your results is made seamless with a variety of options, including to your local data network.

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